Standard Operating Procedure
Litter and Soil Sampling

SOP: 5-1
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Approved: _____ Date Approved: _____

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of soil and litter/manure samples during the Illinois River watershed project.

2.0 Selection of Soil and Litter/Manure Sampling Locations

Sample locations will be selected from either contract growers' farms or company-owned facilities. At each of these farms/facilities, litter from the poultry houses will be collected. Fields where documentation of litter application from a specific farm and Integrator is available from the Oklahoma Department of Agriculture, Food and Forestry will also be selected for sample collection. Field locations selected will be within the Illinois River Watershed. Considerations for sample collection from farms/facilities and fields include:

1. Poultry litter/manure has been consistently generated,
2. Poultry litter/manure is currently being generated,
3. Poultry litter/manure has been consistently (every year for the at least the past 3 years) applied to land (Litter Application Locations, "LALs") associated with the Farm/Facility,
4. Availability of land upon which poultry litter/manure or other fertilizers have not been applied (Control Locations, "CLs").

To the extent possible, the following information should be collected for each associated Farm/Facility:

1. Name of Farm/Facility owner and Farm/Facility contact person,
2. Physical address and location (section-township-range) of Facility,
3. Contact address of Farm/Facility owner or Farm/Facility contact person,
4. Contact phone number of Farm/Facility owner or Farm/Facility contact person,
5. Whether or not one or more LALs can be accessed at the Farm/Facility,

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6. The physical location of each LAL.
7. Whether or not one or more CLs can be accessed at the Farm/Facility.
8. The physical location of each CL.
9. Whether or not a litter/manure and/or nutrient management plan has been prepared for LALs at the Farm/Facility,
10. Estimates of the amounts, rates and dates of prior litter/manure applications to each LAL at the farm/facility,
11. Estimates of litter treatment or amendments added to each LAL (e.g., alum, etc), if any, and information as to amount, rate and dates of application
12. Number, type, dimensions, and capacity of poultry grower houses (or other poultry/egg production facilities, as appropriate) operated at Farm/Facility ("Poultry Houses").

Most of the above information may not be available to the field crews. These data may be acquired through the attorneys during deposition.

3.0 Sampling Documentation

3.1 Sampling Log Book and Sampling Forms

1. Sampling Log Books and/or Sampling Forms will be maintained by the field crews.
2. Pages in the Sampling Log Book will reference specific Sampling Forms by use of the Facility Identification.
3. The Sampling Log Book shall be bound and will be constructed of waterproof paper.
4. Entries in the Sampling Log Book or on the Sampling Form will be made in permanent ink, preferably black ink.
5. Each page of the Sampling Log Book will be dated.
6. The preparer will initial each page of the Sampling Log book.
7. If available, and to the extent possible, for each Farm/Facility sampled, the following information will be recorded in the Sampling Log Book or on the Sampling Forms:
    a. Name, address and phone number of the Farm/Facility owner,

b. Identification of the Farm/Facility, FAC1 – FAC8,

c. Name, address and phone number of the Farm/Facility operator,

d. Name, address and phone number of the Integrator responsible for the Farm/Facility,

e. Names, addresses and phone numbers of persons who have spread litter/manure on LALs associated with the Farm/Facility,

f. The amounts, rates and dates of prior litter/manure applications to specific LALs at the Farm/Facility (confirm State Reports),

g. The existence of prior soil sampling data for LALs or CLs at the facility (yes or no),

h. The water supply for the Farm/Facility,

i. The legal description (qtr-qtr-qtr-sec-twp-rng) of the property related to the Farm/Facility,

j. The legal description (qtr-qtr-qtr-sec-twp-rng) of the CLs at the Farm/Facility,

k. Type of animals generating litter (broilers, layers, pullets, turkeys, etc.),

l. Number of flocks of birds that have used the litter that is sampled,

m. The number of birds in each flock that have used the litter that is sampled,

n. The time since birds last used the litter,

o. Litter treatment (e.g. alum amendment), if any, and information as to amount, rate and date or dates of treatment,

p. Information as to any other fertilizers, chemicals or soil amendments added during the last five years,

q. Use of each LAL by cattle (yes or no) and typical number of cattle,

r. Specific information listed within this protocol,

s. Sketch map of each LAL with approximate dimensions; indicate local features on the sketch (vegetation, water bodies, adjacent fields, location of poultry houses, roads, old fence rows, livestock feeding areas, livestock grazing areas, etc); dimensions and features can also be placed on the aerial photographs,

t. Additional information such as identified springs, wells, seeps, or sinkholes should be indicated on the sketch map or aerial photograph

u. Land slope of each LAL (or LAL sub-area),

v. Distance to nearest water body,

w. Notes on weather (temperature, wind, last precipitation event, etc),

x. Type of vegetation currently on the LAL, if any, and any known vegetation grown in past 5 years,

y. Use of adjacent fields, and;

z. Other information as appropriate or relevant.

3.2 Photographic Record

A photographic record shall be made and maintained for all sampling activities on the LAL. Pictures of the LALs, CLs and the outsides of the poultry house will be taken. No pictures of sampling activities inside the poultry houses will be taken. A video recording will be made, to the extent possible, from a vantage point immediately outside the poultry house.

All photographs made shall be time and date stamped.

3.3 Chain-of-Custody

A Chain-of-Custody will be prepared for each set of samples transferred to the soil and litter processing lab (CDM Support Laboratory in Denver, Colorado). A second chain-of-custody will be prepared at the processing lab for the analytical laboratory.

The Chain-of-Custody to the soil processing lab shall, at a minimum, contain the following information:

1. The project name, ***Illinois River Watershed Soil and Litter/Manure Sampling,***
2. Name of person or entity relinquishing the sample and was part of the field crew,

3. Signature blocks with dates and times for all persons having custody (sampler, shipper, processing laboratory, etc),
4. For each sample related to a Chain-of-Custody:
    a. The unique numeric identifier on the submitted sample container/bag,
    b. The date and time interval the samples were collected,
    c. The sample "matrix" (i.e. SOIL or LITTER or WATER).

4.0 Soil Sampling

4.1 Litter Application Locations (LALs) and Control Locations (CLs)

4.1.1 Permissible Soil and Weather Conditions

1. Soils are not to be sampled if water saturated.
2. Soils are not to be sampled during precipitation events.

4.1.2 Division into Sampling Areas

A Sampling Area is an area within a LAL or CL that is reasonably homogenous with respect to soil types, soil properties, topography, landscapes, management history (to the extent known), and other relevant factors, as appropriate.

1. For each LAL or CL sampled, the LAL or CL shall be divided into a maximum of four Sampling Areas, identified as A, B, C and D.
2. Sampling Areas identified within the LAL or CL shall be a minimum of approximately one acre and shall not exceed approximately 10 acres.
3. In making determinations concerning the division of the LAL or CL into Sampling Areas, the person or persons making those determinations shall consult the USGS topographic map, aerial photograph, and/or other data including relevant USDA/NRCS soil survey. The data consulted shall be identified by reference in the Sampling Log Book.
4. The person or persons who make the determinations concerning the division of the LAL or CL into Sampling Areas shall prepare a sketch map of the LAL or CL and its constituent Sampling Areas. This sketch map shall show the approximate boundaries of each Sampling Area and the estimated area of each Sampling Area. This information can also be recorded on the aerial photographs.

4.2 Identification of Sub-Sampling Locations

A Sub-Sampling Location is a one to ten acre area within a Sampling Area at which individual soil samples will be collected. A total of 20 sub-sampling locations shall be sampled for each Sampling Area. The selection of Sub-Sampling Locations shall avoid:

1. Old fence rows,
2. Livestock feeding areas,
3. Livestock loafing areas, and;
4. Localized conditions atypical of the Sampling Area.

The geographic coordinates (Latitude and Longitude) of the first Sub-Sampling Location in each Sampling Area or a corner of the Sampling Area shall be determined using a Global Positioning System (GPS) receiver accurate to at least five (5) meters. These geographic coordinates will be recorded in the Sampling Log Book.

Representative Sub-Sampling locations will be documented with a time and date stamped photograph.

The following procedure will be used, when possible, to establish a grid system for each Sampling Area (Subareas A, B, C, or D).

1. On the map/aerial photo, select the general area to establish a grid pattern of twenty sampling locations. If the field configuration permits, the Subarea should be either a square or rectangle in shape.
2. The grid setup in either a square or rectangle shape will have 4 evenly spaced sample points within a width and 5 evenly spaced sample points within a length. In other words, the grid system will typically be a 4 by 5 grid, with sample points at the nodes.
3. If the selected grid location is near a fence line or tree line, the corner should be established by insetting a distance of Width (W) of Subarea grid divided by 8 (W/8).
4. Once the corner is established, determine the spacing of the remaining width (W) grid points by dividing the remaining width (RW) by 3. RW equals W – W/4. In summary, width grid points will be established at W/8 and then at distances of (W-W/4)/3.
5. For the length of the Subarea grid, the spacing of the length (L) grid points will be the remaining length (RL) divided by 4. Keeping in mind that the inset distance was W/8, RL equals L – W/4. In summary, length grid points will be established at W/8 and then at distances of (L-W/4)/4.

6. If the Subarea grid is more than 50 feet from a fence or tree line within a field, the grid spacing is simply W/3 by L/4.
7. If the field configuration does not permit the use of rectangular or square Subarea field configurations, try to establish a grid that provides for relatively uniform spacing within the field shape.
8. Record the grid spacing in the field book and, if possible, on the aerial photograph or map.
9. Once the grid spacing has been determined on the aerial photograph or map, the field crew shall use the maps to establish grid layout in the field. Conditions permitting, the grid points will be marked with pin flags, which will be removed after the grid point is sampled.

4.3 Soil Samples to be collected at each Sub-Sampling Location

For purposes of this Protocol, a Sub-Sampling Location shall be an area defined by a triangle with three-foot sides with the middle placed on the Sub-Sampling Location. When possible, one point of the triangle will be oriented in the north direction.

At each Sub-Sampling Location, core samples with a length of at least six inches will be collected at the corners of the triangle. The samples will be divided into three separate soil samples as follows:

1. Four (4) to Six (6) Inch Sample depth. This two inch sample will be collected by measuring the length of the core from the top of the sample. The two inch section of core will be placed in a plastic bag with the appropriate identification.
2. Two (2) to Four (4) Inch Sample depth. This two inch sample will be collected by measuring the length of the core from the top of the sample. The two inch section of core will be placed in a plastic bag with the appropriate identification.
3. Zero (0) to Two (2) Inch Sample depth. This two inch sample will be collected by measuring the length of the core from the top of the sample. The two inch section of core will be placed in a plastic bag with the appropriate identification.
4. One core sample will be collected at each corner of the triangle until enough sample is collected (approximately 100 to 200 grams, depending upon QA/QC needs). The first core will be at the triangle corner oriented to

the north. Additional cores, if necessary, will be collected at the remaining triangle corners.

5. The soil samples collected at each sub-sampling location will be collected with soil probe coring devices either marked for 6-inch, 4-inch and 2-inch depths, or with a vertical slot so that the core measurements can be made with a ruler. The diameters of all soil corers used should be the same, and should be of a diameter consistent with general practice for agricultural soil sampling.
6. Whenever a soil sample is to be collected, thatch and other plant residue shall be moved aside or lightly scuffed aside without removing the surface soil prior to pushing the soil probe core into the soil.
7. Coring devices will be manually driven to at least six inches in depth if possible. If coring devices are being driven with a post hole driver and the coring device shows no or very limited advancement after ten consecutive blows, the coring device will be considered to have reached refusal. The corer shall be extracted and the available core collected. Attempts to collect soil samples from the missing core depths can be made at the remaining triangle corners.
8. Core recovery will be noted for each 2-inch interval. Recovery will be qualified as good, poor, or no recovery. Poor recovery will note that an incomplete two-inch sample was recovered.
9. In the event that soil conditions do not permit the use of a soil probe coring device, samples may be collected with a shovel.
10. Thatch and other plant residue shall be removed prior to collecting a sample with a shovel.
11. When a shovel is used for collection the following procedure shall be followed:
    a. At each sub-sampling location, dig a hole at least 6 inches deep.
    b. During excavation, material from zero to 2 inches should be placed in a bag appropriately labeled for the depth. Then the material from 2 to 4 inches should be placed in a separate bag. And finally the material from 4 to 6 inches should be placed in a separate bag.
    c. Material from each depth interval may be placed on a plastic sheet to facility sample collection.

When possible, representative soil samples collected from the field will be documented with a time and date stamped photograph.

4.4 Handling of Samples

All individual samples from each sub-location will be placed in individual plastic bags. The sample number will be placed on the outside of the sample bag. Each sampling area (up to four sampling areas per LAL) will have 60 individual samples (20 sub-sample locations x 3 sample depths = 60 samples). For each subarea, the sample bags from each depth will be segregated and placed in a larger resealable reinforced plastic bag (typically one-gallon freezer bags). For example, all zero to 2-inch samples within sub-location "A" will be placed in the same one gallon resealable plastic bag. Soil samples from one LAL or CL will then be placed within one large plastic bag which will be sealed before it is placed in an insulated container (cooler). All samples will be shipped to the soil/litter processing laboratory for compositing.

Compositing of samples will be performed at the soil processing laboratory.

4.5 Field QA/QC Samples (Soils)

1. Field Duplicate Samples may be created at the soil processing lab.
2. Blind Standard: A blind standard of a certified reference soil may be sent to the analytical lab for every 50 samples send to the analytical laboratory. The blind standard will be sent by the soil processing lab.
3. Decontamination Blank: a sample of the final decontamination rinsate may be collected and forwarded to the soil processing laboratory for analysis at a frequency of one decon rinsate collected after sampling is completed at a facility or at a rate of one per 20 decontamination events . The decon blank will be generated in the field using laboratory grade distilled water.

4.6 Decontamination Procedures

Full decontamination will occur between every LAL property, or upon exit of a growers' field onto a public right-of-way. A decontamination station will be established and maintained at the boundary of the grower's property and the public right-of-way, unless a location has otherwise been designated by the grower or integrator.

Full decontamination steps will be as follows:

1. Bagged samples will be placed into a receiving bag held by the members of the decon team.
2. All electronic equipment will be transferred from the resealable plastic bags carrying the electronic equipment into the field into a plastic bag held by a member of the decon team.
3. All reusable tools will be decontaminated by removing all soil or other material by brushing/scraping the equipment. The equipment will then be washed with a phosphate free soap solution. This will be followed by a rinse a 6 or 10 percent bleach solution and then with distilled water.
4. All disposable PPE equipment such as gloves, coveralls, boot covers, etc. will be removed and disposed into a plastic trash bag held by the decon team. The trash bag will be placed into a second trash bag and tied shut.
5. The rubber boots worn by the field crews will then be decontaminated using the same procedures used to decontaminate the reusable tools. Upon decontamination of the rubber footwear, the field crew members may leave the field.
6. Any vehicles driven onto the LAL fields will be driven through the decon line with the front tires brushed to remove soil and other material, sprayed and brushed with a phosphate free detergent solution, and then sprayed with a bleach solution. Once the front tires and wheel wells have been decontaminated, the rear tires will be addressed using the same procedure before the vehicle enters the public right-of-way.

Decontamination between subareas within an LAL and not requiring Full Decontamination procedures will consist of removing soil material from the corer barrel and the knife or implement used to cut the soil samples prior to collection of the first soil sample from the next LAL subarea.

After discussion with Oklahoma Department of Agriculture, Food, and Forestry personnel, it was determined that the decontamination water and solutions will be considered *de minimus* material and will be disposed of on the ground on the right-of-way leading into the facility.

5.0 Litter/Manure Sampling

5.1 General Conditions

1. All litter/manure samples will be collected with litter/manure in place within Poultry Houses.

2. Litter/manure may be sampled at any time regardless of weather conditions.
3. More than one Poultry House may be sampled at a Farm/Facility. The litter/manure from each house will be maintained as a separate sample.
4. Bio-Security Protocol dictated by the Oklahoma Department of Agriculture, Food, and Forestry, and as supplemented by individual integrators and/or growers will be followed at all times.
5. The sampling team will consist of three individuals. One individual will enter the Poultry House and collect the samples. A second individual will accompany the first individual onto the property but will only video tape the first individual from a vantage point generally outside of the Poultry House. The third individual will maintain their position at a decontamination station anticipated to be at the public right-of-way entrance to the grower's property.
6. The individual responsible for the video taping will relay house entry times, house exit times, start of compositing times, and completion of sample compositing times to a third individual located at the public right of way entry to the grower's property via radio communications. The third individual will enter those times into the field book.
7. Prior to entry onto the grower's property, a decontamination/ sample handling station will be established on the public right-of-way adjacent to the grower's property, or on the grower's property if an adequate location is identified by the grower.

5.2 Location and Distribution of Poultry House Sub-Sample Collection Points

1. Broiler or Pullet Houses
   a. Sub-samples are collected from approximately 1/3 house-width zones.
   b. Approximately six samples are collected from each zone.
   c. Sub-samples should be located so as to obtain two samples from around the waters, feeders and walls on each side of the house.
   d. Depending upon the size of the poultry house, sub-samples are estimated to be spaced at 20 to 25 pace intervals within each 1/3 zone.

e. Sub-sampling locations alternate between the "sides" of each zone (i.e. a "zig-zag" pattern is traversed between sampling locations within a zone).

f. Sub-samples collected from adjacent zones should not be immediately adjacent.

2. Breeder Houses (partially slatted)

a. Sub-samples will be collected from both slatted and litter areas.

b. Twenty (20) sub-samples will be collected

c. Sub-samples will proportionally represent the relative aerial proportion of slatted and litter areas; for example if 2/3 of the house is under slats, and 1/3 is litter area, 14 litter/manure samples should be collected from under the slats and 7 litter/manure samples should be collected from the litter area.

d. Sub-samples taken beneath slats will be as fully penetrating of the manure as possible and will be distributed so as to obtain a representative sample of the entire slatted area.

e. Sub-samples from litter areas will be collected in the same manner (i.e. "zig-zag" pattern) as used for broiler or pullet houses.

3. Other Circumstances

a. Sampling of litter/manure within a Poultry House for circumstances and conditions other than those described for Broiler, Pullet or Breeder Houses will be conducted so as to obtain a representative sample of the litter/manure within that Poultry House.

b. The circumstances or conditions requiring a variation from the sampling protocol described for Broiler, Pullet or Breeder Houses will be documented in the Sampling Log Book.

c. A description of the method(s) and procedures used to collect a representative sample of the litter/manure within a Poultry House in which the sampling protocol for Broiler, Pullet or Breeder Houses cannot be followed will be documented in the Sampling Log Book.

d. The method(s) and procedures used to collect a representative sample of the litter/manure within a Poultry House in which the sampling protocol for Broiler, Pullet or Breeder Houses cannot be

followed will follow the principles embodied in the reference materials.

e. All sub-samples will be collected with an appropriate solid manure sampling device.

f. All samples from litter areas will be collected through the full thickness (surface to base) of the litter/manure.

g. All samples from slatted areas will, to the extent possible, be collected through the full thickness (surface to base) of the litter/manure.

h. Immediately after collection, all sub-samples will be placed in a plastic bag contained inside a 5-gallon plastic bucket.

i. For partially slatted houses, sub-samples from slatted and litter areas will be composited together.

4. Container

a. During sample collection, all samples will be placed into a 5-gallon bucket double-lined with plastic bags.

b. After sample collection, the material within the 5-gallon bucket will be manually mixed using either a clean hand trowel and/or the shovel used to collect the samples inside the poultry house.

c. The rough mixing/compositing will be accomplished by breaking the cake material and turning over, to the extent possible, the entire contents of the bucket without damaging the plastic bag liners.

d. The rough mixing/compositing will be conducted immediately outside the poultry house and immediately after sample collection.

e. After mixing, a small subsample (500 ml in volume) will be removed via hand trowel and placed into a sterile plastic bottle or whirl pack, which will be immediately sealed and labeled with the Sample ID.

f. The remaining material within the plastic bags lining the 5-gallon bucket will be tied shut. At the decon station, the plastic bags will be placed into another appropriately sized plastic bag which will be tied shut, sealed with duct tape, and the sample ID written on the duct tape with an indelible marker.

g. The small subsample container will also be placed into a resealable plastic container at the decon station. This second container will also be labeled with the sample ID and date using an indelible ink marker.

h. The samples will then be placed in an appropriately-sized cooler with ice that will also be double bagged in plastic bags and sealed shut.

i. The appropriate chain-of-custody will be placed inside each cooler and the cooler sealed with tape and a chain of custody label.

j. The cooler with the 500 ml volume sample will be shipped overnight directly to the EML Lab for bacteria analyses.

k. The cooler with the remaining litter sample will be shipped overnight to the soil/litter processing lab.

5. Exiting the Property

a. Once the field team has sampled a Poultry House, the team will approach the decontamination station maintained at the boundary of the grower's property and the public right-of-way, unless a location has otherwise been designated by the grower or integrator.

b. Samples will be handed across the decon station line into clean receiving bags as noted above.

c. All electronic equipment will be passed from the sampling team into a resealable plastic bag held by the decon team member. The electronic equipment will be wiped down with an antibacterial wipe followed by a cloth moistened with dionized water.

d. Sample trowels, shovels, and empty collection bucket will be offered to the grower. If the grower does not want these tools, they will be included with the protective coveralls and gloves to be discarded to a sanitary landfill or a municipal incinerator.

e. All disposable Personal Protective Equipment (PPE) to be disposed will placed into double bagged plastic bags held by the decon team member. These bags will be disposed at a dumpster serviced by a municipality that either disposes of the trash at a sanitary landfill or by incineration.

f. The rubber boots of the sampling team will be subject to decontamination by a phosphate free detergent rinse, followed by a bleach solution rinse, followed by a tap water rinse.

g. Once the boots have been decontaminated, the sampling team may cross the decontamination line onto the public right-of-way.

5.5 Field QA/QC Samples (Manure/Litter)

1. Field Duplicate Samples may be created in the soil/litter processing lab.
2. Decontamination Blank (created in the field): a sample of the final decontamination rinsate may be collected and forwarded to the processing lab to send to the analytical lab for analysis at a frequency of one decon rinsate for every facility. A decon rinsate would only be generated in the event that sampling equipment were to be reused. Currently, the plan is that all sampling equipment for manure/litter sampling is disposed after a single use.

6.0 Identification of Samples

Identifying information to be recorded on the sample label for soil samples:

1. Alphanumeric identification of the LAL or CL: LAL1 – LAL24, CL1 – CL8. The log book will be used to record the farm and location of each LAL or CL.
2. Alphanumeric identification of the Sampling Area: A – D
3. Alphanumeric identification of the Sub-sample location: 1 - 20
4. Alphanumeric identification of the depth of collection (i.e. –2, -4, -6)
5. The following sample number is an example of the soil sample taken from LAL field number 5, sampling area B, sub-sample location 18, and a depth of 2 inches:

   LAL5-B-18-2

6. For samples submitted to the analytical lab, additional alphanumeric identification of the type of sample will be added to the end of the identification number:
   a. A= laboratory sample
   b. B = laboratory duplicate
   c. C = reference soil (standard)

d. D = decontamination blank (added to field samples)

e. E = laboratory QA/QC (extra volume)

7. Date of sample collection (only on chain-of-custody),
8. Time of sample collection (only on chain-of-custody),
9. Initials of the person collecting the sample (only on chain-of-custody).

6.1 Identifying information to be recorded on the sample label for litter/manure samples:

1. Alphanumeric identification of the Facility: FAC1 – FAC8.
2. Alphanumeric identification of the Poultry House: A – C
3. The following sample number is an example of the litter sample taken from facility number 5 and poultry house B:

   FAC5-B

4. Samples sent to the analytical laboratory will have alphanumeric identification of the type of sample added to the end of the number:
   a. A= laboratory sample
   b. B = laboratory duplicate
   c. C = reference soil (standard)
   d. D = decontamination blank (added in the field)
   e. E = laboratory QA/QC (extra volume)
5. Date of sample collection (only on chain-of-custody),
6. Time of sample collection (only on chain-of-custody),
7. Initials of the person collecting the sample (only on chain-of-custody).

7.0 Shipment of Samples to the soil/litter processing laboratory and to the analytical laboratory

1. Once placed in sampling containers (plastic bags or jars), samples will be placed on ice (double bagged and sealed in plastic bags) within insulated protective containers.
2. If possible, samples will be shipped immediately via overnight shipment to the analytical laboratory.

3. In no event, will FAC samples be held more than 24 hours before shipment. Depending upon circumstances, LAL samples may be held as much as 48 hours before shipment.
4. Samples will be sent to the laboratory under a Chain-of -Custody.
5. A custody seal will be placed on the outside of the container across the area between the lid and the container. The custody seal will be signed.
6. The Chain-of-Custody will be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.
7. Samples shipped to the EML laboratory will be shipped to the following address:

   Environmental Microbiology Laboratory
   1150 Bayhill Drive, Suite 100
   San Bruno, CA 94066
   Contact: Cole Mackelprang, 858-268-2762
   e-mail: cmackelprang@emlab.com
   Contact: Megan S. Tatreau, 858-268-2770

8. Samples shipped to the CDM Prep laboratory will be shipped to the following address:

   CDM
   2714 Walnut Street
   Denver, CO 80205
   Contact: Todd Burgesser, 303-298-1311
   e-mail: burgesserte@cdm.com

8.0 Analytical

8.1 Laboratory

The laboratory conducting the analyses will be experienced in conducting the specified analyses and will have certifications to conduct the specified analyses.

All analyses and sample preparation will be conducted using accepted and published protocols and/or methods.

8.2 Analytical Protocols

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 1 provides the parameters and analytical methods for the short and Table 2 provides the parameters and analytical methods for the long list.

Litter samples will be analyzed for Table 2 parameters.

Table 1: Short List Parameters – Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Nitrogen	Kjeldahl, modified
Total Aluminum (Al)	EPA SW-3050/6020
Total Phosphorus (P)	EPA SW-3050/6020
Total Arsenic (As)	EPA SW-3050/6020
Total Copper (Cu)	EPA SW-3050/6020
Total Zinc (Zn)	EPA SW-3050/6020

Table 2: Long List Parameters – Manure and Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Texture (% sand, silt and clay)	Hydrometer ASTM-D422
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Phosphorus (P)	EPA SW-3050/6020
Mehlich-III Phosphorus (Mehlich-III P)	Mehlich III (ICP)
Soluble Phosphorus	Water 1:10, Bull.396, pg 17
Soluble nitrate	Water 1:10
Total Nitrogen	Kjeldahl, modified
Soluble ammonium	Water 1:10
Soluble sulfate	Water 1:10
Soluble chloride	Water 1:10
TAL Metals	EPA SW-3050/6020
Total Molybdenum (Mo)	EPA SW-3050/6020

Bacteria:	
Total coliform	SM-9221B
Enterococcus	SM-9230B
Fecal coliform	SM-9221E
e-coli	SM-9221F
Staphylococcus	BAM 12
Campylobacter	BAM-Chap. 7
Salmonella	BAM 5
17β-estradiol, estrone, estriol	LC-MS-MS

8.3 Data Reporting

1. Data from the laboratory shall be reported in both electronic and paper reports.
2. Data reports shall include all quality control data generated, including results for duplicates, blanks and spikes, as applicable. If applicable, a level 3 data quality report will be provided by the laboratory.
3. Data reports shall include a copy of the Chain of Custody accompanying each set of samples submitted

9.0 Bio-security, Decontamination of Equipment and Personal Protective Equipment

All persons engaged in sampling, observing sampling or documenting sampling under this protocol shall follow appropriate bio-security precautions. All persons doing sampling will receive bio-security training from the State of Oklahoma.

9.1 Soils

To the extent possible, disposable sampling equipment should be used.

All reusable sampling equipment shall be decontaminated using a non-phosphate detergent, a 6% (minimum) bleach solution, and three de-ionized water rinses between Sampling Areas.

9.2 Litter/Manure

To the extent possible, disposable sampling equipment should be used.

All reusable sampling equipment shall be decontaminated using a non-phosphate detergent, a 6% (minimum) bleach solution, and three de-ionized water rinses between poultry houses.

9.3 Health and Safety Plan:

A health and safety plan that is specific to this sampling protocol will be prepared and reviewed by all samplers.

10.0 References

Zhang, H. and Johnson, G. 2003. How to get a good soil sample. Oklahoma State University Cooperative Extension Service Fact Sheet F-2207. Available at http://osuextra.okstate.edu/pdfs/F-2207web.pdf

Zhang, H., Hamilton, D. W. and Britton, J. G. 2002. Sampling Animal Manure. Oklahoma State University Cooperative Extension Service Fact Sheet F-2248. Available at http://osuextra.okstate.edu/pdfs/F-2248web.pdf

Eucha/Spavinaw Watershed Management Team. Undated. Soil Sampling Protocol.

Eucha/Spavinaw Watershed Management Team. Undated. Steps for Pulling Litter Samples.

11.0 Revised Dates*

The following are other revision dates applicable to this SOP.

Revision 8 – February 5, 2007
Revision 7 – April 24, 2006
Revision 6 – May 11, 2005
Revision 5 – April 20, 2005
Revision 4 – March 25, 2005
Revision 2, 3 – March 16, 2005
Revision 1 – January 25, 2005

SOP: 5-2
Standard Operating Procedure
Litter and Soil Sample Compositing

Revision: 3*
Initial Date: 05/03/05
Last Revised: 02/05/07
Page 1 of 7

Prepared: Todd Burgesser Review: Kim Zilis

Approved: Roger L. Olsen Date Approved: 2/06/07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for compositing of soil and poultry litter samples from the Illinois River watershed of eastern Oklahoma and western Arkansas. This will include handling, mixing, and shipment of soil and litter samples.

2.0 Handling and Compositing of Soil and Litter Samples

All individual soil samples from each sub-location will be placed in individual plastic bags (double bagged), packed in a cooler with blue ice and shipped over night under chain-of-custody to the CDM processing laboratory in Denver, Colorado. The sample number will be located between the inner and outer plastic bag. Each sampling area (up to four sampling areas per LAL) will have 60 individual samples (20 sub-sample locations x 3 sample depths = 60 samples). All samples will be received by the CDM processing laboratory for compositing. Each of the 20 sub-samples will be composited into one homogeneous sample using the protocol described below.

Litter samples will be received by the CDM processing laboratory under chain-of custody in a 5-gallon bucket. The litter sample will be contained in a plastic bag inside of the 5-gallon bucket will be closed with a tie. A unique sample number will be written on the outside of the bucket.

Upon receipt of the samples, the cooler/bucket temperature will be measured using a NIST traceable thermometer. The samples soil will then be removed from the cooler and checked against the chain-of-custody to ensure that all samples have been received.

The twenty sub-samples associated with the individual sample depths or the entire litter sample will be poured into a stainless steel bowl or 2.5-gallon bucket ready for mixing. All equipment will be decontaminated/sterilized with laboratory grade distilled water and 10 percent bleach (see procedure below).

2.1 Mixing of Soil Samples

- All health and safety protocol will be followed as described in the Health and Safety Plan for the Illinois River Basin Project. This is includes wearing nitrile gloves and processing soil in the hood.
- All feathers, rocks, twigs, debris and vegetation will be removed before sieving and mixing.
- Mixing will be accomplished using a disposable, plastic sampling scoop or a decontaminated stainless steel spoon.
- All clods over 0.5 inches in diameter will be disaggregated into smaller particles by hand or the use of a decontaminated stainless steel spoon or mortar.
- If the moisture content is too high to allow homogenization or disaggregation of the particles, the sample will be placed in steel drying pan and air dried over night.
- The sample will be hand mixed using the plastic scoop or stainless steel spoon for at least five minutes or until particles are uniform in size.
- If a plastic bucket is used, the bucket will then be sealed and inverted or rotated at least 10 times.
- After mixing, the sample will be sieved to remove particles sizes of greater than 2 mm using a decontaminated US Sieve no. 10 (gravel size particles will be removed).
- Each fraction (greater than 2 mm and less than 2 mm) will be weighted. The less than 2 mm fraction will be placed in a plate grinder and reduced in size to 0.074 mm (US sieve no. 200, very fine sand).
- The ground sample will be split using a riffle splitter and sent to the various laboratories (see splitting procedure in section 1.3.1, Duplicate Samples).

2.2 Mixing of Litter Samples

The same procedure as described above for the soil will be used for the litter. However, grinding may not be necessary if the litter can be sieved directly through a US sieve no. 200.

2.3 Laboratory QA/QC Samples (Soil)

Laboratory QA/QC samples may consist of duplicate samples, decontamination blanks, and blind standards. The following describes each type of QA/QC sample.

2.3.1 Duplicate Samples (created at the soil processing lab)

After sample mixing, sieving and grinding, two split samples will be collected. The sub-sample splits should be collected using a nonbiased riffle splitter. The sample is poured through the riffle splitter and into the decontaminated collection pans. The amount of soil or litter contained by the sample container shall be sufficient for the chemical and physical analyses to be conducted.

2.3.2 Blind Standards

A blind standard of a certified reference soil will be sent to the analytical laboratory for approximately every 50 samples send to the laboratory. The blind standard will be sent by the CDM soil processing lab. Blind standards will be for metals, arsenic, and phosphorus.

2.3.3 Decontamination Blanks

A sample of the final decontamination rinsate will be collected and forwarded to the analytical laboratory for analysis. The decontamination rinsate blank will be generated in the CDM processing laboratory using a final rinse of laboratory grade distilled water. All parameters will be analyzed.

3.0 Shipment of Samples to the Analytical Laboratory

- Once placed in sampling containers (plastic bags or jars), samples will be held at 4° C on blue ice (sealed in plastic bags) within insulated protective containers.
- If possible, samples will be shipped immediately after compositing via overnight shipment to the analytical laboratory.
- After compositing, samples should not be held more than 24 hours before shipment.
- Samples will be sent to the laboratory under a Chain-of -Custody.
- A custody seal will be place on the outside of the cooler between the lid and the body of the cooler. The custody seal will be signed.
- The Chain-of-Custody will be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.

4.0 Decontamination of Processing Equipment

All nondisposable equipment (bowls, sieves, spoons, and grinders) will be decontaminated/sterilized after each composite sample is created. Decontamination will include washing with phosphate free water followed by rinsing with laboratory grade distilled water. A final rinse of 10 percent bleach will be performed. The equipment will be air dried.

5.0 List of Analytes and Bottle Requirements

5.1 Analytical Parameters

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 1 provides the parameters and analytical methods for the short list and Table 2 provides the parameters and analytical methods for the long list.

Litter samples will be analyzed for Table 2 parameters.

Table 1: Short List Parameters - Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Nitrogen	Kjeldahl, modified
Total Aluminum (Al)	EPA SW-3050/6010/6020
Total Phosphorous (P)	EPA SW-3050/6010/6020
Total Arsenic (As)	EPA SW-3050/6010/6020
Total Copper (Cu)	EPA SW-3050/6010/6020
Total Zinc (Zn)	EPA SW-3050/6010/6020

Table 2: Long List Parameters – Manure and Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Texture (% sand, silt and clay)*	Hydrometer ASTM-D422
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Phosphorous (P)	EPA SW-3050/6020
Mehlich-III Phosphorous (Mehlich-III P)	Mehlich III (ICP)

Soluble Phosphorous	Water 1:10, Bull.396, pg 17
Soluble nitrate	Water 1:10
Total Nitrogen	Kjeldahl, modified
Soluble ammonium	Water 1:10
Soluble sulfate	Water 1:10
Soluble chloride	Water 1:10
TAL Metals	EPA SW-3050/6020
Total Molybdenum (Mo)	EPA SW-3050/6020
Bacteria:	
Total coliform	SM-9221B
enterococcus	SM-9230B
Fecal coliform	SM-9221E
e-coli	SM-9221F
staphylococcus	BAM12
campylobacter	BAM7
salmonella	BAM5
17β-estradiol, estrone, estriol	LC-MS-MS

*split before sieving and grinding

5.2 Bottle Requirements

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 3 provides the parameters, bottle requirement and laboratory for the short list and Table 4 provides the parameters, bottle requirement and laboratory for the long list.

Litter samples will be analyzed for Table 4 parameters.

Table 3: Short List Parameters - Soil

Parameter	Bottle	Laboratory
Moisture content (%)	1 quart glass	A&L
Organic matter	1 quart glass	A&L
Soil pH	1 quart glass	A&L
Soil Conductivity	1 quart glass	A&L
Total Nitrogen	1 quart glass	A&L
Total Aluminum (Al)	1 quart glass	A&L
Total Phosphorous (P)	1 quart glass	A&L
Total Arsenic (As)	1 quart glass	A&L
Total Copper (Cu)	1 quart glass	A&L
Total Zinc (Zn)	1 quart glass	A&L

Note: 1 bottle for all of the above analysis

Table 4: Long List Parameters – Manure and Soil

Parameter	Bottle	Laboratory
Moisture content (%)	1 quart glass	A&L
Organic matter	1 quart glass	A&L
Texture (% sand, silt and clay)*	1 quart glass (separate from the other bottles)	A&L
Soil pH	1 quart glass	A&L
Soil Conductivity	1 quart glass	A&L
Total Phosphorous (P)	1 quart glass	A&L
Mehlich-III Phosphorous (Mehlich-III P)	1 quart glass	A&L
Soluble Phosphorous	1 quart glass	A&L
Soluble nitrate	1 quart glass	A&L
Total Nitrogen	1 quart glass	A&L
Soluble ammonium	1 quart glass	A&L
Soluble sulfate	1 quart glass	A&L
Soluble chloride	1 quart glass	A&L
TAL Metals	1 quart glass	A&L
Total Molybdenum (Mo)	1 quart glass	A&L
Bacteria	1 - 250 mL plastic (sterilized) or 1-8 oz. Whirl bag	EML
PCR	1-8 oz. Whirl bag	ISU
17β-estradiol, estrone, estriol	1 – 4oz. glass	GEL

*split before sieving and grinding

6.0 Analytical Laboratories

Bottles for estrogen metabolites (all samples) will be shipped to:

General Engineering Laboratories, LLC
201 Pine Ridge Road, Unit 5
Golden, CO 80403
Contact: Paul Winkler, 720-253-3093
Paul.winkler@gel.com

Bottles for nutrients, metals, etc (all samples) will be shipped to:

A&L Analytical Laboratories, Inc.
2790 Whitten Rd.
Memphis, TN 38133
Contact: Scott McKee, 800-264-4522
smckee@allabs.com

Bottles for bacteria analyses from soil and litter will be shipped to:

Environmental Microbiology Laboratory
1150 Bayhill Drive, Suite 100
San Bruno, CA 94066
Contact: Megan S. Tatreau, 858-268-2770
mtatreau@emlab.com

Bottles for PCR will be shipped to:

Idaho State University
Department of Biological Sciences-MRCF
Attn: Erin O'Leary-Jepsen
640 Memorial Drive
Pocatello, ID 83209-8007
Contact: Erin O'Leary-Jepsen, 208-282-4890

7.0 Documentation

Bound laboratory logbooks should be used for the maintenance of field records. All aspects of sample compositing and handling as well as visual observations will be documented in the field logbooks. Supplemental information may be documented on the field data sheets provided. All entries in laboratory logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

8.0 Revised Dates*

The following are other revision dates applicable to this SOP.

Revision 2: 02/09/06

Revision 1: 05/10/05

Standard Operating Procedure
Manure Sampling for DNA Analysis

SOP: 5-3
Revision: 2*
Initial Date: 4/26/2006
Last Revised: 2/6/2007
Page 1 of 9

Prepared: Darren L. Brown Review: Roger Olsen

Approved: Date Approved: 2-06-07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of fecal matter for identifying the types and abundance of bacterial DNA. The bacterial DNA is first amplified by polymerase chain reaction (PCR), then digested with a restriction enzyme. The enzyme cuts DNA strands into different size fragments whose length is dependent upon the DNA sequence, and the last (terminal) fragment is labeled for detection. Each terminal fragment length is represents approximately one bacterial species. This program is designed to identify DNA fragments from bacteria that reside in fecal material from various animals, including cattle, swine, ducks, geese and humans.

2.0 Selection of Sampling Locations

Sample locations will be selected from farms, wildlife areas, septic clean-out trucks, or wastewater treatment plants as appropriate. The following sources of fecal matter will be targeted for collection.

1. A total of 10 fields where beef cattle are actively grazing; preferably five fields within the basin and five fields outside the basin,
2. A total of 2 dairy cattle milking barns; preferably in the basin, but could be outside of the basin (close to the basin as possible),
3. A total of 2 swine facilities; preferably in the basin, but could be outside of the basin (close to the basin as possible),
4. A total of five active geese landing areas; preferably in the basin, but could be outside of the basin (close to the basin as possible),
5. A total of five active duck landing areas; preferably in the basin, but could be outside of the basin (close to the basin as possible),
6. A total of three septic clean out trucks; preferably all in the basin, but at a minimum at least one sample in the basin,
7. A total of three small wastewater treatment plan influent locations; preferably all in the basin, but at a minimum at least one in the basin.

The locations should contain the following information for each associated Farm/Facility:

1. Name of Farm/Facility owner and Farm/Facility contact person,
2. Physical address and location (section-township-range) of Facility,
3. Contact address of Farm/Facility owner or Farm/Facility contact person,
4. Contact phone number of Farm/Facility owner or Farm/Facility contact person,
5. Whether or not one or more samples can be accessed at the Farm/Facility,
6. The physical location of each sample collection site(s) - record coordinates (latitude and longitude) of documented location (eg, corner of a field),
7. Estimate of number of animals at sample collection site or number of facilities serviced by wastewater treatment plant or septic clean out truck,
8. Estimate of the amount of feces available at the sampling site,
9. Estimate of when the feces was deposited; e.g., was the animal observed while it was defecating,
10. Observation as to whether any chicken litter application has occurred at the sampling field/site,
11. Estimates of amount, rate, and date of litter treatment applied to the site, if applicable, and information as to amount, rate and dates of application.

Site selections will be made based upon availability.

3.0 Sampling Documentation

3.1 Sampling Log Book and Sampling Forms

1. A Sampling Log Book and Sampling Forms shall be maintained.
2. Pages in the Sampling Log Book will reference specific sampling forms by use of the Sample Identification.
3. The Sampling Log Book shall be bound and shall be constructed of waterproof paper.
4. Entries in the Sampling Log Book or on the sampling form shall be made in black permanent ink.
5. Each page of the Sampling Log Book shall be dated.
6. The preparer shall initial each page of the Sampling Log book.

7. For each location sampled, the following information shall be recorded in the Sampling Log Book or on the sampling forms:
   a. Name, address and phone number of the Property/Facility owner,
   b. Identification of the Property/Facility (MAN),
   c. Name, address and phone number of the Property/Facility operator,
   d. If applicable, name, address and phone number of the Integrator responsible for the Property/Facility,
   e. If applicable, the amounts, rates and dates of prior litter/manure applications to specific fields at the Property/Facility (confirm State Reports),
   f. If applicable, the existence of prior soil sampling data for the property (yes or no),
   g. The water supply for the Property/Facility,
   h. The legal description (qtr-qtr-qtr-sec-twp-rng) of the property related to the Property/Facility,
   i. Information as to any fertilizers, chemicals or soil amendments added during the last five years,
   j. Specific information listed within this protocol,
   k. Sketch map of each property/facility with approximate dimensions; indicate local features on the sketch (vegetation, water bodies, adjacent fields, location of poultry houses, roads, old fence rows, livestock feeding areas, livestock grazing areas, etc); dimensions and features can also be placed on the aerial photographs,
   l. Land slope of property/facility,
   m. Distance to nearest water body,
   n. Notes on weather (temperature, wind, last precipitation event, etc),
   o. Type of vegetation currently on the LAL, if any, and any known vegetation grown in past 5 years,

p. Use of adjacent fields, and;

q. Other information as appropriate or relevant.

3.2 Photographic Record

A photographic record shall be made and maintained for all sampling activities on the MAN. All photographs made shall be time and date stamped.

3.3 Chain-of-Custody

A Chain-of-Custody shall be prepared for each set of samples transferred to the analytical laboratory, North Wind, Inc. in Idaho Falls, ID (see section 7).

The Chain-of-Custody shall, at a minimum, contain the following information:

1. The project name, ***Illinois River Watershed Manure DNA Sampling,***
2. Name of person or entity collecting samples,
3. Signature blocks with dates and times for all persons having custody (sampler, shipper, processing laboratory, etc),
4. For each sample related to a Chain-of-Custody:
   a. The unique numeric identifier on the submitted sample container/bag (see subsequent section 6)
   b. The date and time the sample was collected,
   c. The sample "matrix" (Manure).

4.0 Manure Sampling

4.1 Manure Locations (MAN)

4.1.1 Permissible Manure and Weather Conditions

1. Manure must be fresh. Sample should be from the interior of manure piles.
2. Manure should not be sampled during precipitation events.

4.1.2 Beef Cattle Sampling Areas

Manure samples will be collected from a total of ten fields actively grazed by cattle. Five locations will be from fields within the IRW. If available, both fields with and without litter application will be sampled. Five locations will be from fields outside the IRW and, if

possible, from fields with no litter application. Two composite samples will be collected from each field. Each composite sample will consist of samples from ten fresh manure piles. In all, twenty composite samples will be collected.

4.1.3 Dairy Cattle Sampling Areas

Manure samples will be collected from the clean out slurry of four milking barns. If possible, two barns handling cattle fed by grazing and two barns handling grain-fed cattle will be sampled. The clean out slurry must consist of that day's droppings. The samples must be collected from waste stream before the collection ponds. In all, four samples will be collected.

4.1.4 Swine Sampling Areas

Manure samples will be collected from the clean out slurry from two swine facilities. The clean out slurry must consist of that day's droppings. The samples must be collected from waste stream before the collection ponds. In all, two samples will be collected.

4.1.5 Duck Sampling Areas

Manure samples will be collected from up to five landing or residence areas. Sampling locations will be from wildlife areas, golf courses, or local ponds. Two composites will be collected from each landing/residence area. Composites will consist of ten swabs or direct fecal samples each, if possible. In all, ten samples will be collected.

4.1.6 Geese Sampling Areas

Manure samples will be collected from up to five landing or residence areas. Sampling locations will be from wildlife areas, golf courses, or local ponds. Two composites will be collected from each landing/residence area. The locations may be co-located with the duck locations; however, the samples have to be distinctly separate between species. Composites will consist of ten fecal samples each, if possible. In all, ten samples will be collected.

4.1.7 Human Waste Samples

Human sewage samples will be collected at two sources: septic clean out trucks and influent to wastewater treatment plants. Sewage samples will be collected from three separate septic clean out trucks. The samples should be collected at the pump out facility after at least several homes have been visited. The sample should be collected after the pumping has been in progress and the waste in probably mixed.

Sewage samples will be collected from the plant influent at three different wastewater treatment plants. The plant operator will determine the best way to collect a representative influent sample which has not been subject to treatment. Wastewater treatment plants will be selected that do not have contribution from industries which could contribute poultry or other animal waste products (i.e. processing plants).

In all, six human waste samples will be collected.

4.2 Collection and Handling of Samples

Sampling personnel will wear disposable, sterile gloves at all times when collecting fecal samples and will change gloves before they collect each new fecal sample. Samples will either be pre-composited samples (i.e. dairy cattle, swine, and human samples) or will be composited in the field (beef cattle, duck, and geese). All samples will be collected into 20 milliliter, sterilized, polystyrene, round bottom tubes. Each tube will contain 10 mL of 20 % glycerol solution (added to the tube by the laboratory). Pre-composited samples will be collected directly into the tubes (approximately 2 - 10 grams). For the samples to be composited in the field, ten aliquots will be sampled using a sterilized, disposable, polystyrene spatula. A similar sized sample (1 -2 grams) from each individual stool will be placed into one tube. The contents will then be mixed in the field by shaking the tube containing the glycerol/waste mixture. If swabs (sterile, cotton-tipped applicators) are used to collect duck feces, all the swab tips (ten) will be placed into the same round bottom tube. The tips will be cut from the attached plastic tube (or stick) using scissors (sterilized by cleaning with an alcohol wipe before use). Labels will be placed on the tubes and secured with transparent tape. The tubes will be placed inside individual resealable plastic bags. The bags will be placed in a cooler containing dry ice before leaving the property/facility where the sample was collected. The samples must be frozen prior to being shipped to the analytical laboratory. If the samples have not been frozen by exposure to the dry ice, they shall be placed in a freezer until freezing is complete. Samples will remain frozen until immediately prior to shipping. Samples shall be placed in a cooler with standard ice and shipped priority overnight to the analytical laboratory.

4.3 Field QA/QC Samples (Manure)

1. Duplicates: no field duplicate samples will be created since samples will be composite samples.
2. Blind Standard: no blind standards will be submitted for this particular program.
3. Decontamination Blank: no decontamination blanks will be generated for this particular program as all collection equipment will not be reused between samples.
4. Field Blanks: field blanks will be collected at a rate of one per twenty or per sample shipment. Field blanks will be collected by one of three methods.
    a. Dairy Cattle, Swine, and Humans - one field blank associated with one of these locations will be collected by opening the screw top cap and immediately replacing the cap. The tube will contain the glycerol from the laboratory.

b. Beef Cattle and Geese – one field blank associated with one of these locations will be collected by opening a packet containing a sterilized collection spatula and placing it directly into the screw cap tube containing the glycerol.

c. Duck – one field blank associated with one of these locations will be collected by placing a swab tip directly into the screw cap tube containing the glycerol.

4.4 Decontamination Procedures

Sampling equipment will be one time use. No equipment decontamination is anticipated. Only the scissors will be reused and these will be cleaned with an alcohol wipe between sampling sites.

If appropriate, bio-security decontamination measures will be implemented. All waste generated during the sampling procedure will be placed in disposable trash bag and placed in a container where the waste will be transported to a sanitary landfill.

5.0 Person(s) Collecting Samples and Observing Sampling

Personnel from CDM or Lithochimeia will conduct the manure sampling from each MAN. CDM personnel will process samples, chain-of-custody, coordinate shipping, etc.

6.0 Identification of Samples

Identifying information to be recorded on the sample label for DNA Manure samples:

1. Beef Cattle: Alphanumeric identification will consist of MAN-BC-1, MAN-BC-2 etc. The log book will be used to record the facility/property and location of each composite sample.
2. Dairy Cattle: Alphanumeric identification will consist of MAN-DC-1, MAN-DC-2 etc. The log book will be used to record the facility/property and location of each composite sample.
3. Swine: Alphanumeric identification will consist of MAN-SW-1, MAN-SW-2 etc. The log book will be used to record the facility/property and location of each composite sample.
4. Duck: Alphanumeric identification will consist of MAN-DK-1, MAN-DK-2 etc. The log book will be used to record the facility/property and location of each composite sample.
5. Geese: Alphanumeric identification will consist of MAN-GS-1, MAN-GS-2 etc. The log book will be used to record the facility/property and location of each composite sample.

6. Human: Alphanumeric identification will consist of MAN-HM-1, MAN-HM-2 etc. The log book will be used to record the facility/property and location of each composite sample.
7. If necessary, an alphanumeric identification will be assigned to a subarea if more than one sample is collected from the same facility/property: A, B, C, D etc.
8. The following sample number is an example of a manure sample taken from Beef Cattle field number 5, sampling area B:

   MAN-BC-5-B

9. For samples submitted to the analytical lab, additional alphanumeric identification of the type of sample will be added to the end of the identification number:

   a. F = Field Blank

10. Date of sample collection (only on chain-of-custody),
11. Time of sample collection (only on chain-of-custody),
12. Initials of the person collecting the sample (only on chain-of-custody).

7.0 Shipment of Samples to the analytical laboratory

1. Shipping coolers will be packed such that samples are stored with standard ice placed in double-bagged resealable plastic bags. The shipping coolers shall be insulated protective containers.
2. If possible, samples shall be shipped immediately via overnight shipment to the analytical laboratory. The laboratory address is:

   Idaho State University

   Department of Biological Sciences- MRCF
   Attn: Erin O'Leary-Jepsen

   650 Memorial Drive
   Pocatello ID 83209-8007
   208-282-4890

3. In no event, shall samples be held more than 24 hours before shipment unless they are frozen.
4. Samples shall be sent to the laboratory under a Chain-of -Custody.
5. A custody seal will be place on the outside of the container across the area between the lid and the container. The custody seal will be signed.

6. The Chain-of-Custody shall be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.

8.0 Analytical

8.1 Analytical Protocols

Analyses are being conducted by Tamzen W. Macbeth (208-528-8718), North Wind, Inc., 1425 Higham St., Idaho Falls, ID 83402. Analytical protocols are provided in a separate document.

8.3 Data Reporting

1. Data from the laboratory shall be reported in both electronic and paper reports.
2. Data reports shall include all quality control data generated, including results for duplicates, blanks and spikes, as applicable.
3. Data reports shall include a copy of the Chain of Custody accompanying each set of samples submitted

9.0 Bio-security, Decontamination of Equipment and Personal Protective Equipment

All persons engaged in sampling, observing sampling or documenting sampling under this protocol shall follow appropriate bio-security precautions.

9.1 Manure

To the extent possible, disposable sampling equipment should be used.

Any reusable sampling equipment shall be decontaminated using a non-phosphate detergent, bleach and three de-ionized water rinses between Sampling Areas. No reusable equipment is currently anticipated.

9.2 Health and Safety Plan:

The overall health and safety plan for the project will be used for this sampling protocol and will be reviewed by all samplers.

10.0 Revised Dates*

The following revision dates are applicable to this SOP:

Revision 1 -July 11, 2006